

イノベーションに情熱を。  
ひとに思いやりを。



## 第一三共グループにおける研究開発 生産性向上の取組み

2012年10月24日

第3回JSPS研究開発専門委員会

春山英幸

第一三共RDノバレ株式会社

# 第一三共の2015年ビジョン

2015年に第一三共グループが目指す企業像  
Global Pharma Innovator の実現

事業エリア拡大への挑戦

Global

世界の国々に  
自らが拠点を構えて  
自ら事業を展開する企業

アンメットメディカルニーズ  
への挑戦

Pharma

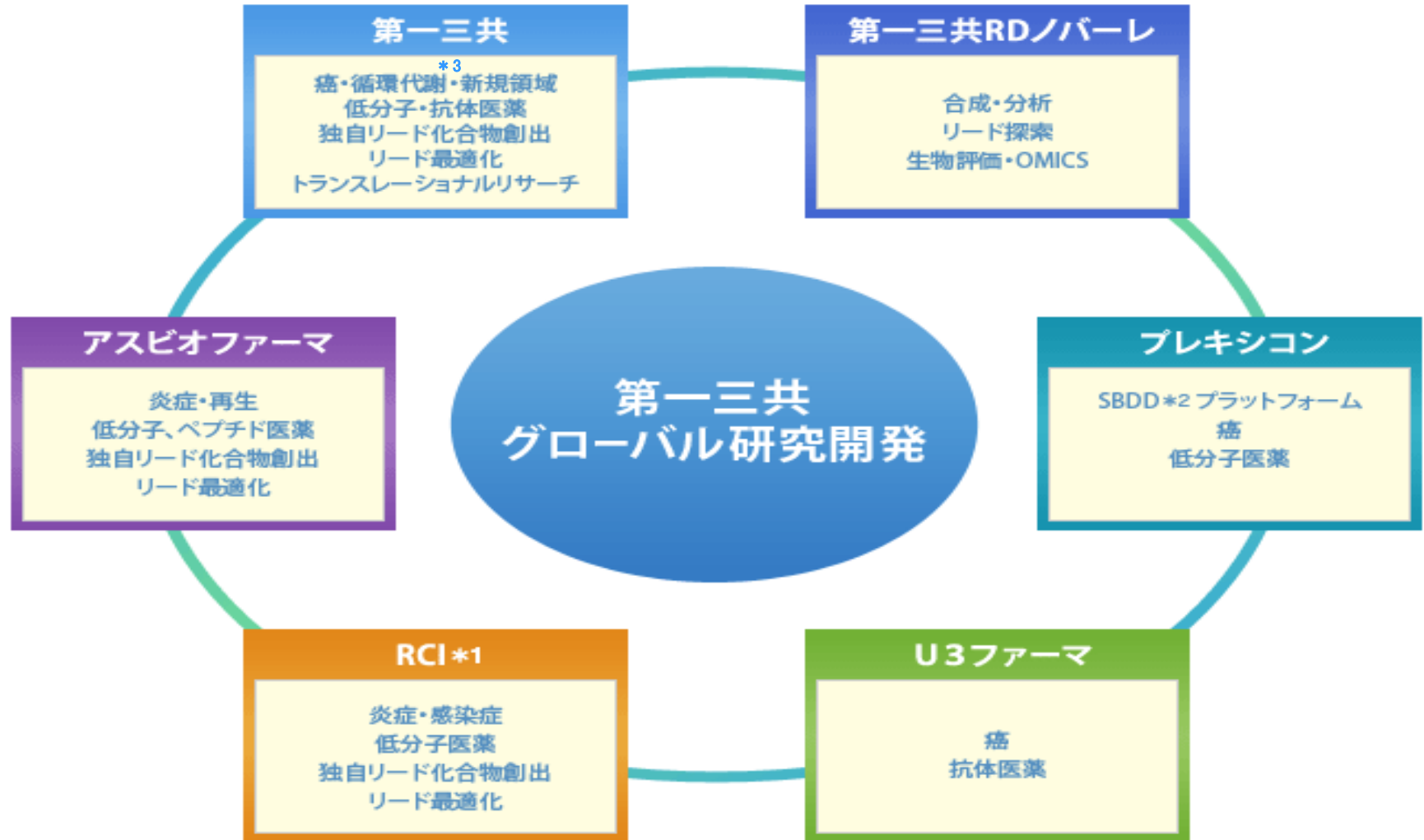
経営資源を医薬品に  
集中し、革新的医薬品を  
継続的に創出し、  
多様な医療ニーズに応える  
医薬品を提供する企業

新たなビジネス  
モデル構築への挑戦

Innovator

サイエンス・技術における  
イノベーションのみならず  
ビジネスモデルの  
イノベーションを  
実現する企業

# グローバル研究機能



\*1 RCI : Daiichi Sankyo Life Science Research Centre in India

\*2 SBDD: Scaffold-Based Drug discovery

\*3 循環代謝: 心臓・血管系疾患に関わる代謝性疾患

# 重点領域の絞り込み

## Key Message

未充足医療ニーズの高い重点領域での競争力の向上

2010-  
(第2期以降)

「重点」カテゴリー

癌

循環代謝

「新規」カテゴリー

未充足ニーズへの  
新たなチャレンジ  
FIC\*に特化

狙い

2015年に向けて現在の基盤の上に  
さらなる競争力を構築する

2015年以降に向けて  
従来の疾患領域に  
とられない新たな  
切り口でチャレンジする

2007-09  
(第1期)

血栓症、癌、糖尿病、自己免疫/関節リウマチ

\*FIC: First in Class

# RD ノバーレは、第一三共グローバルRDの共通創薬基盤プラットフォームとして発足

2011年4月

## 研究開発本部

- 疾患領域に特化した機能  
癌、循環代謝、先端医薬

- 共通基盤技術機能;  
ADME, Tox/ HTS/ 蛋白発現/  
天然物/オミックス技術

## RD アソシエ;

### 研究開発支援機能

化学合成/分析業務/生物評価/  
臨床開発 (モニター業務)

- プロセスを集約することで習熟効果が働き、効率・生産性が上がる
- 分離することのデメリットに比してメリットが大きい
- プログラム/プロジェクト固有の技術で無い
- 活動のアウトプットが、プロダクトのIPそのものではない

2011年10月

## 研究開発本部

- 疾患領域に特化した機能;  
癌、循環代謝、先端医薬

- 共通基盤技術機能;  
前臨床研究

## RD ノバーレ; FIC創薬のためのソリューションプロバイダー

- 初期探索研究のための統合技術プラットフォーム
- 臨床開発運営プラットフォーム (POC取得以降)

## 創薬基盤技術のライフサイクル

黎明期

萌芽期

発展期

汎用期

成熟期

再編期

科学的  
検証

創薬応用  
検証

実践使用

汎用  
標準化

標準化  
効率化

改廃

Technology

ウォッチング

技術の育成  
・基盤整備

技術の標準化  
・集約化

技術の標準化  
・効率化・高度化

技術の選択  
・集中・外注化

第一三共RDノバーレのコア技術

# ヒト組織中の薬物代謝酵素同定のための proteomic correlation profilingの応用

Proteomic correlation profiling to identify a drug-metabolizing enzyme  
in human tissue

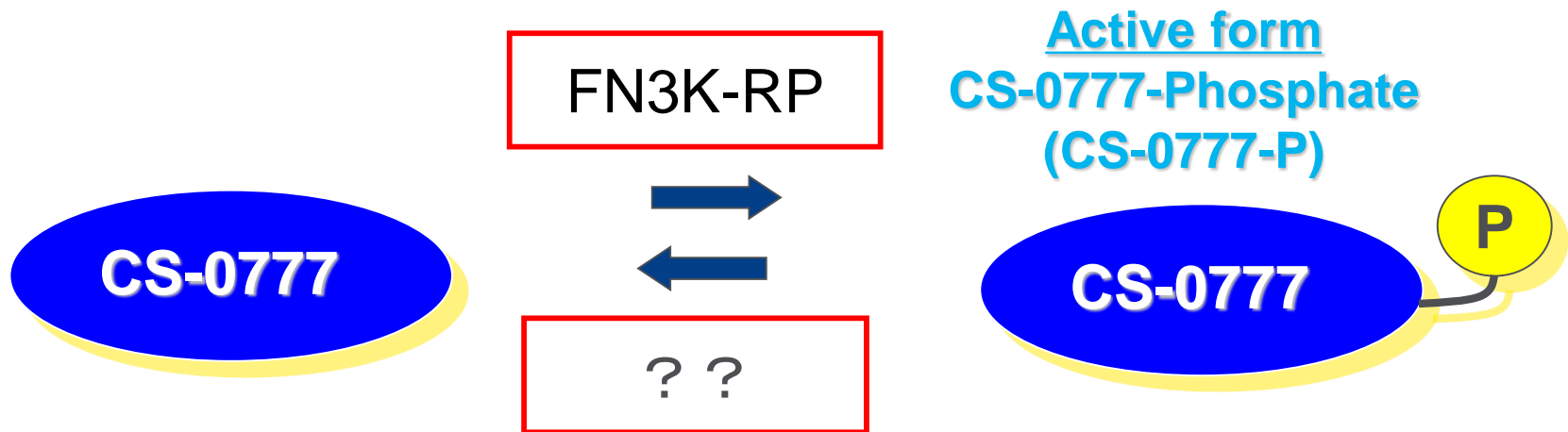
Hedetaka Sakurai<sup>1</sup>, Kazuishi Kubota<sup>1</sup>, Shin-ichi Inaba<sup>2</sup>,  
Kaoru Takanaka<sup>2</sup>, Akira Shinagawa<sup>1</sup>

<sup>1</sup>DAIICHI SANKYO RD NOVARE CO., LTD, <sup>2</sup>DAIICHI SANKYO  
CO., LTD

日本プロテオーム学会2012年年会  
2012年7月26、27日

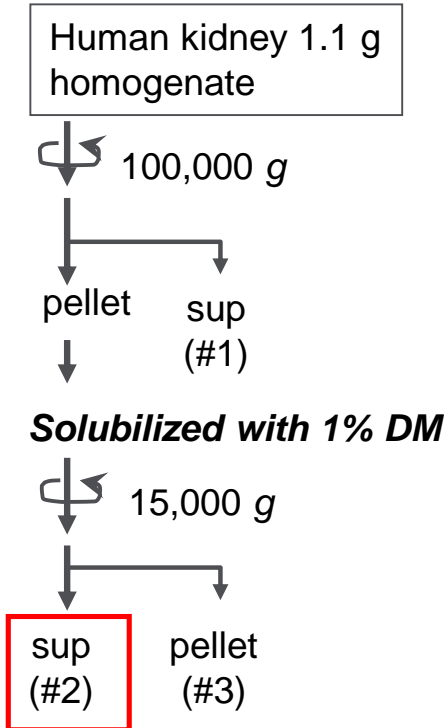
# Introduction

- CS-0777 and Fingolimod need phosphorylation to have a S1P<sub>1</sub> agonist activity.
- Fingolimod was approved as a first oral drug for multiple sclerosis by FDA in September 2010.
- The kinase and the phosphatase for Fingolimod have been already reported.
- The kinase for CS-0777 identified by our team is different from a Fingolimod kinase.
- **The phosphatase for CS-0777 has not been identified so far.**
- Identification of the phosphatase is required to fully understand mechanism of activation and inactivation of CS-0777.

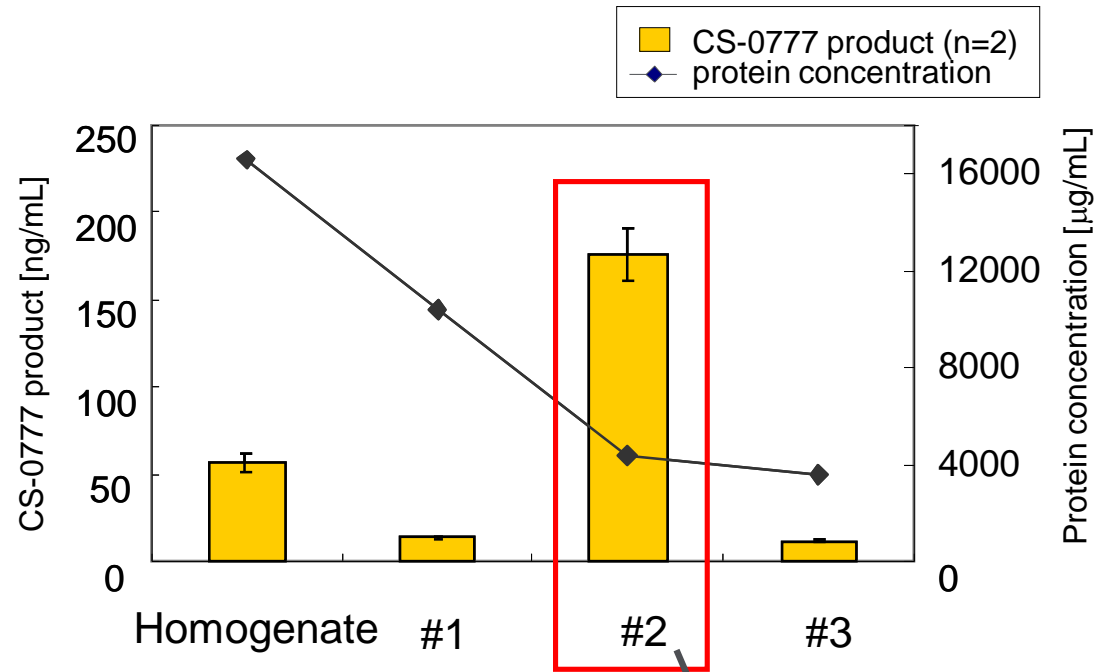




# Purification - 1



Fractionated human kidney homogenate



- CS-0777-P phosphatase activity mainly resided in the insoluble fraction.
- A detergent (1% DM) was found to be very effective in solubilizing the activity.

**Used for purification**

# Purification - 2

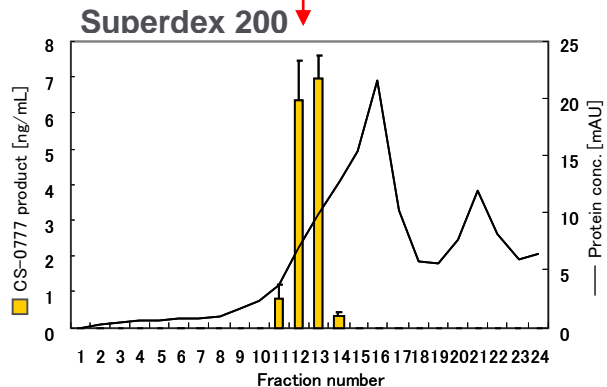
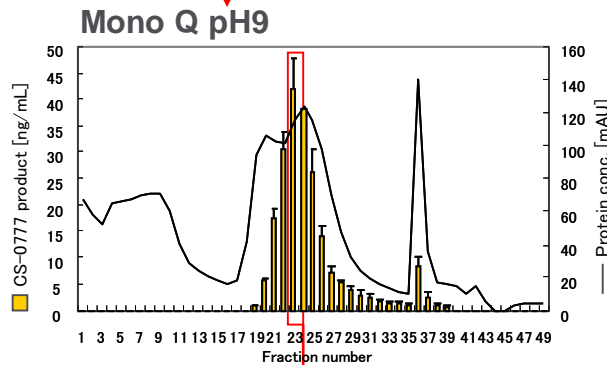
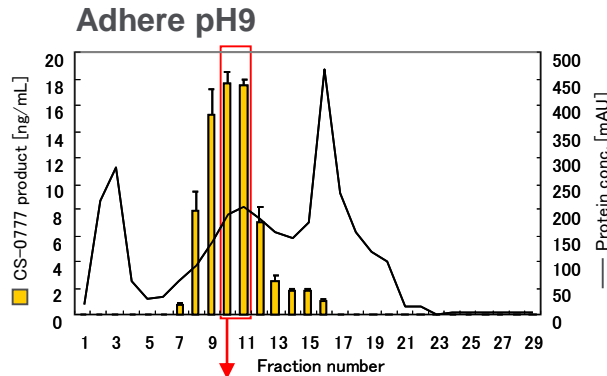
#2 fraction obtained from Purification - 1

↓  
**Step 2**  
Multimodal anion-exchange  
**Adhere pH9**

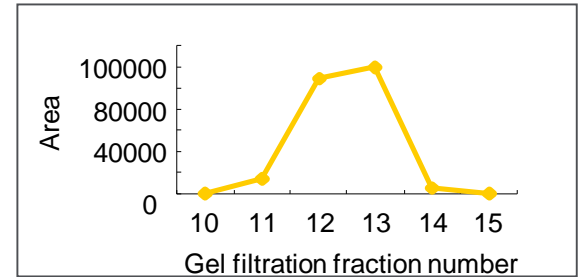
↓  
**Step 3**  
Anion-exchange  
**Mono Q pH9**

↓  
**Step 4**  
Gel Filtration  
**Superdex 200**

Enzyme activity was concentrated by about 200-fold.



### Phosphatase activity



*We were not able to find the band that correlated with CS-0777-P phosphatase activity by this purification.*

### Gel stain



# Identification of candidate proteins by Proteomic Correlation Profiling\*

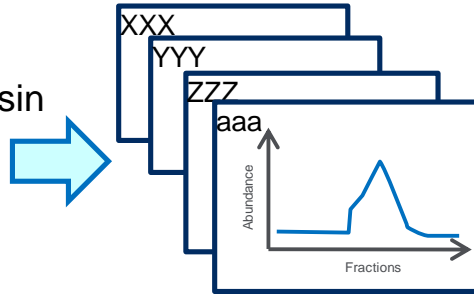
Gel filtration of the enzyme active fractions



Proteins digested by trypsin

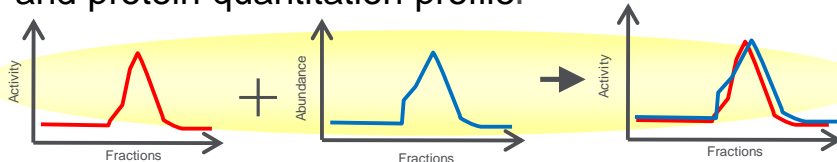
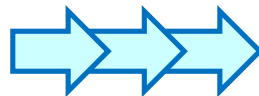
LC-MS/MS

Database search



Identified **274** proteins and MS-based protein quantitation

Draw profile comparisons phosphatase activity profile and protein quantitation profile.



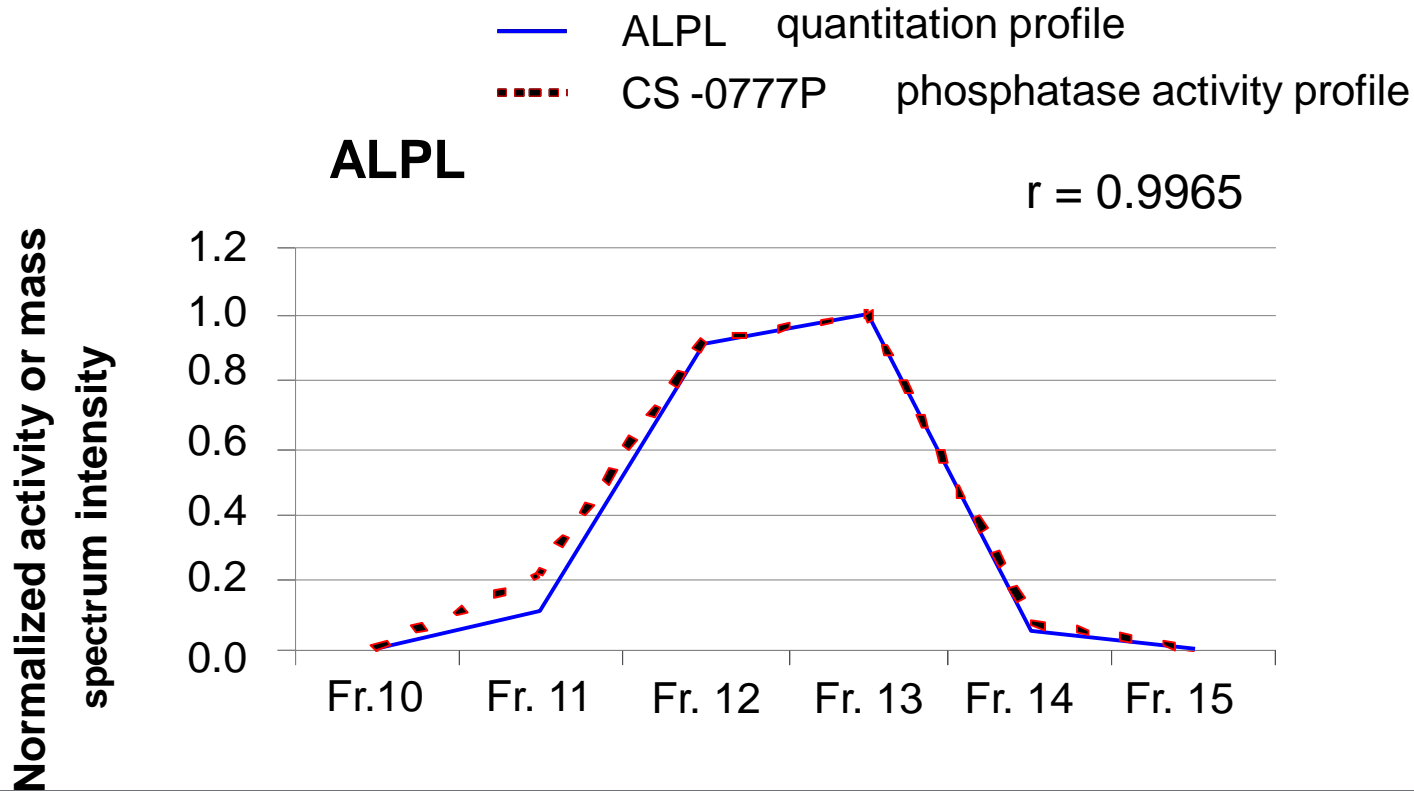
Rank	Gene symbol	Correlation coefficient ( $r$ )
1	SLC13A2	0.9984
3	SLC2A1	0.9958
4	NCSTN	
5	SLC22A6	
6	SLC22A8	0.9835
7	SLC17A3	0.9817
8	SLC22A12	0.9742
9	HADHA	0.9741
10	FH	0.9739
11	BSG	0.9682
12	LAMP1	0.9277
13	HADHB	0.9226
14	SLC5A12	0.9182
15	XPNPEP2	0.8871
16	DPP4	0.8855
17	C9orf46	0.8673
18	MME	0.8513
19	ATP1B3	0.7816
19	SLC22A12	0.7816

**Rank 2 / 274**

The Pearson correlation between the protein amount and CS-0777-P phosphatase activity for all 274 proteins was calculated.

\* Kubota K. et al. (2009) *Nat. Biotechnol.* **27**, 933-940

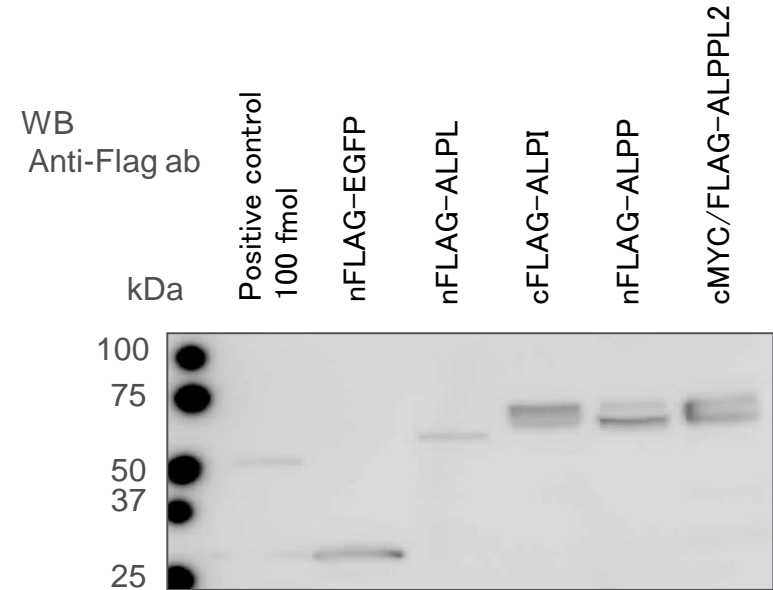
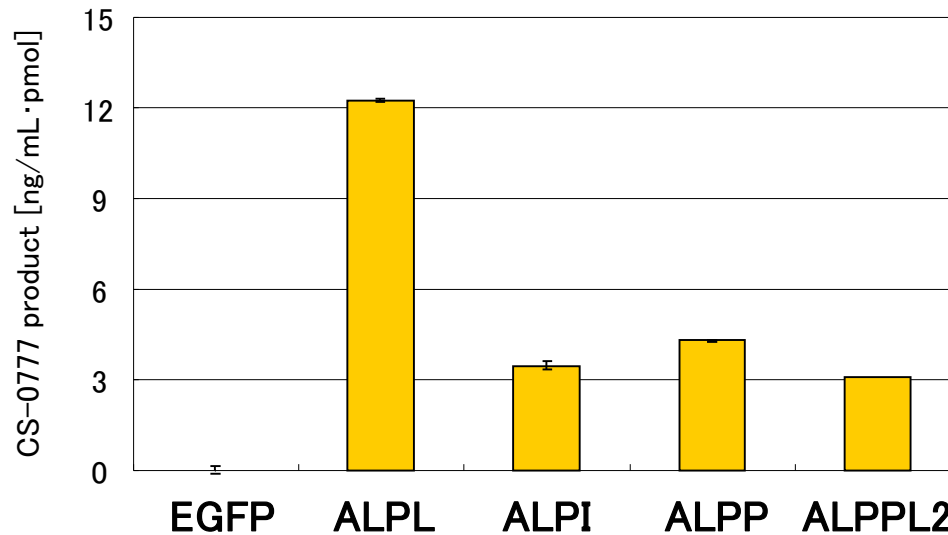
# Identification of candidate proteins by Proteomic Correlation Profiling



Alkaline phosphatase activities for , tissue none specific isozyme (ALPL) showed best correlation between phosphatase activity and MS-based quantitation as phosphatases.

# CS-0777-P phosphatase activity of recombinant ALP isozymes

## Specific enzyme activity for recombinant ALP isozymes



## Recombinant all ALP isozymes had CS-0777-P phosphatase activity !

In particular, ALPL had 3-fold higher specific activity compared to other ALP isozymes.

**ALPL:** Tissue-nonspecific isozyme (Liver/Bone/Kidney), **ALPI:** Intestinal isozyme, **ALPP:** Placental isozyme, **ALPPL:** Placental like isozyme (Germ cell)



Daiichi-Sankyo

# Gene Targeting Discovery of Novel Nucleoside Antibiotics

*Gordon Research Conference, Natural Products*  
**July 22-27, 2012**

**Masanori FUNABASHI<sup>1</sup>, Satoshi BABA<sup>2</sup>, Toshio TAKATSU<sup>3</sup>, Masaaki KIZUKA<sup>1</sup>,  
Masahiro TANAKA<sup>1</sup>, Steven G. Van Lanen<sup>4</sup>, Koichi NONAKA<sup>5</sup>**

<sup>1</sup> Natural Product Research Group, Discovery Science and Technology Department, Daiichi Sankyo RD Novare Co., Ltd., Japan

<sup>2</sup> Biologics Research Laboratories, R&D division, Daiichi Sankyo Co., Ltd., Japan

<sup>3</sup> Analytical Chemistry Research Group, Center for Pharmaceutical and Biomedical Analysis, Daiichi Sankyo RD Novare Co., Ltd., Japan

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DAIICHI SANKYO RD NOVARE CO., LTD.

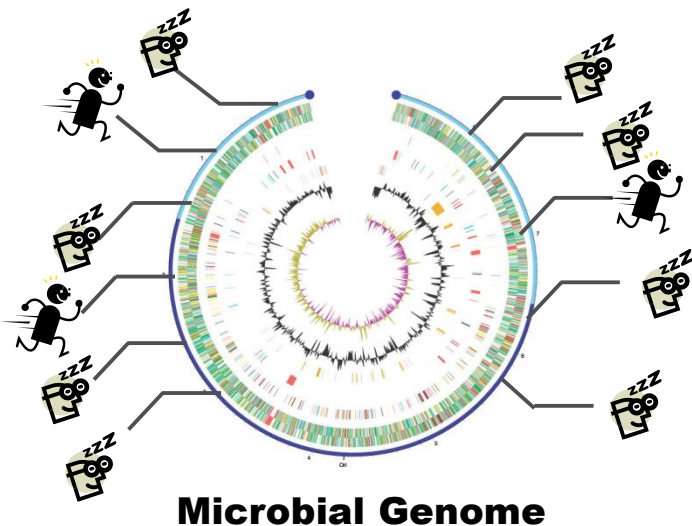
The number of new drug leads from natural products has decreased

Is there a perception that the variety of natural product has run dry?

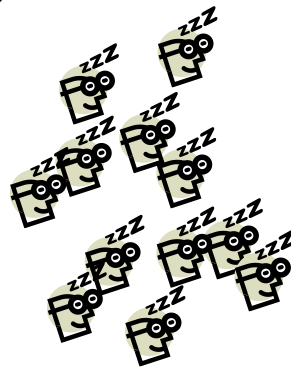


## The Answer is No

From recent microbial genomics research revealed that there is a huge number of **'silent'** biosynthetic gene clusters



**'Silent'**  
Biosynthetic Gene Clusters

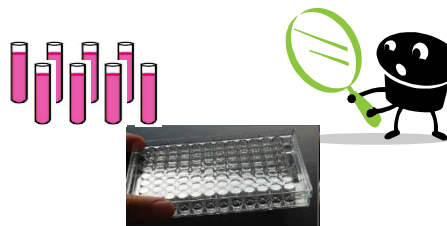


**Treasure House of Drug Leads !**

# Approaches to discover new compounds

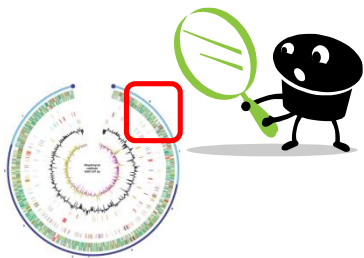
## Traditional Strategy

Bioassay-guided

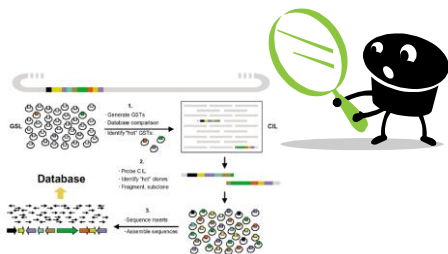


## Emerging Strategy

Genome Mining Approach

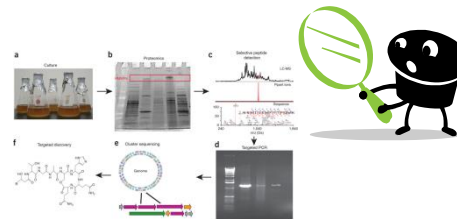


Genome Scanning Approach



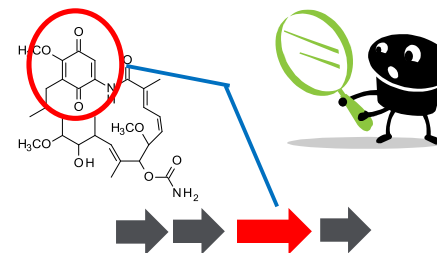
Zazopoulos E., *et al.* Nat. Biotech. 21 187-190

Proteomics Approach



Bumpus S. B. *et al.* Nat. Biotech. 27 951-956

Gene Targeting Approach





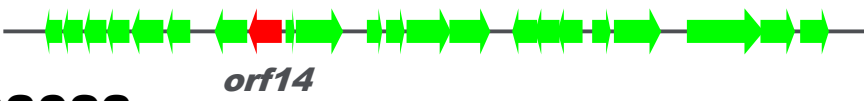
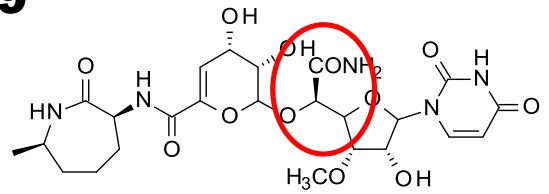
# Motivation

# Gene targeting approach for novel translocase I inhibitors

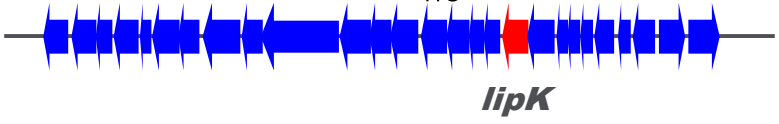
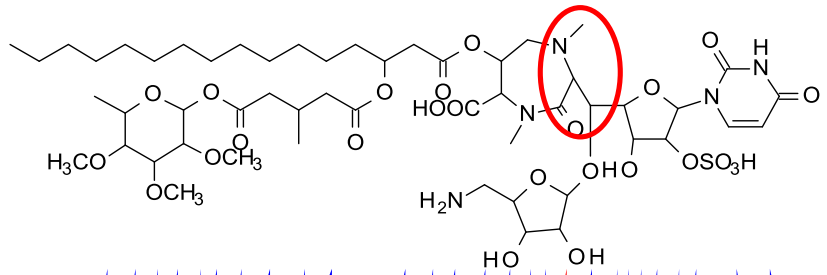
## Hypothesis

We could discover strains which have a potential for producing translocase I inhibitor with uridine moiety by targeting the SHMT gene

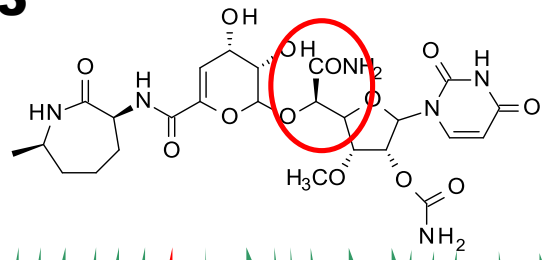
**A-500359**



**A-90289**



**A-503083**



**Serine hydroxymethyltransferase (SHMT) homolog is conserved in the uridine-nucleoside biosynthetic gene clusters (*orf14*, *capH* and *lipK*) is a key enzyme for the biosynthesis of these uridine-nucleoside antibiotic**

# Results

## I. Construction of Degenerate Primers    II. Validation for PCR Condition

```

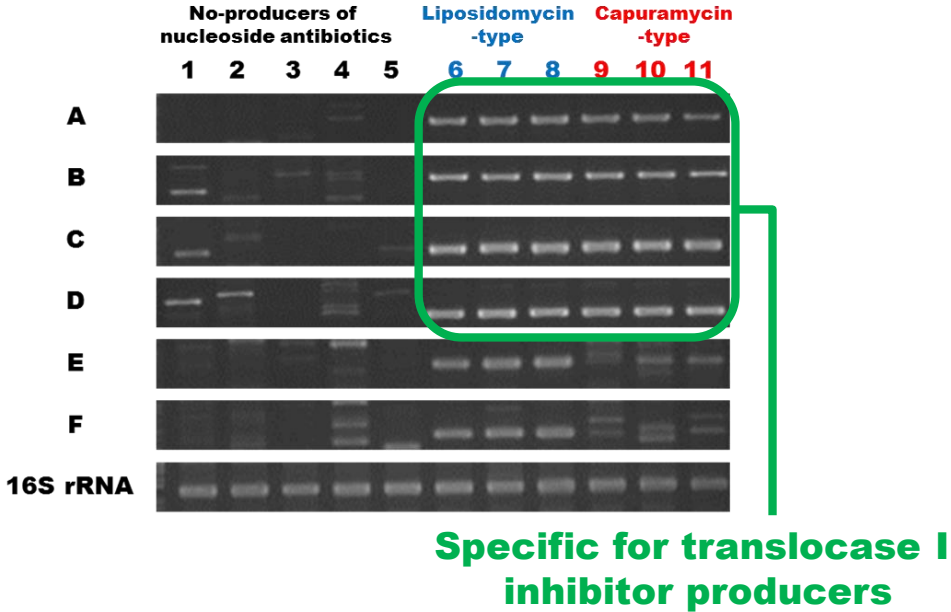
Orf14 (A-500359) 1:MVAQPRTLMIARGAHTHRMPTGLEGRREANVIDFEGSNRRRFPGDRMGTIKELDVV 60
CapH (A-503083) 1:-----MTDIRELRKYV 11
LipK (A-90289) 1:-----MTVGAGGKTSADADPLML 19
.....*
           DS-F1      DS-F2
Orf14 (A-500359) 61:DRFRAEERKAATAVNLVPSNRISFLAQPLSLTDYNNRYFNEDALDPGFWQFRGGQEVAE 120
CapH (A-503083) 12:DRFRAQERKAASINLVPSNRISFLAQMPDLDYNNRYFNEDALDPGFWQFRGGQEVAK 71
LipK (A-90289) 20:RAIADDRRAAHALNLVPSNRISFLASLPLASDFNNRYFNEDGDPDFLWFEFRGGEDIAH 79
.....*
           DS-F3
Orf14 (A-500359) 121:IQTEIARGLHLSRLSRAFHVNERPISGLSMMMLAGLGKPGGTVVSVGAESGGHYATAG 180
CapH (A-503083) 72:IQTEIARGLHSRLRARPVNERPISGLSMMMLAGLGKPGGTVVSDAASGGHYATAD 131
LipK (A-90289) 80:IEA-LGAAALFRMASARYCNVRIPISGMSAMILVVAAL-SFPGSTVVSDGNSGGHYATFA 137
.....*
Orf14 (A-500359) 181:MARRLGFESATVPVAHQVDEQRLQQLLRERTPQLLYDLQNSRHELEVSRAELIKEYS 240
CapH (A-503083) 132:MARRLGFESATVPVVRGVDEQWFQVLRHVPPELVLDLQNSRHELEVSRAELIEAHS 191
LipK (A-90289) 138:LLGLRHSRLLNCKDGEVDESLEAELVAGDVALVYVUVQNCVVPDFRMSDVIREV 197
.....*
           DS-R1
Orf14 (A-500359) 241:PTTLLHVDCHTMGLLGLSALGNPLDAGADTMGSGTHKTFQPHKGVLFTRSPELHQRLK 300
CapH (A-503083) 192:PHILHVDCHTMGLLGLSALSNPLDAGAHMGTGTHKTFQPHKGVLFTRSPELHQRLK 251
LipK (A-90289) 198:PGTRIVDASHYLGLVGLLANPLDGGADAFGGSTHKSFFQPHKGVIFRNAEDVDESIR 257
.....*
           DS-R2
Orf14 (A-500359) 301:DAQFTMLSSHFAETLSLGLAAAEFHFFGQVAEQQVIANRLESFLAADDGFDVAADENG 360
CapH (A-503083) 252:HAQFTMLSSSHFAETLALGLAAAEFHHFQVAVYAEQQVIAHRLGLLAAADDGFDVADENG 311
LipK (A-90289) 258:SAQFDLVSSSHFAETLALSLAALEVEDRMGDYRATNDNARLAGALADGFRVYGSAT 317
.....*
Orf14 (A-500359) 361:HATSTHQVWVKIGDAERTDRISQALYEHGIRVNVQVDLPLGPPALRLGVNLTFTGGRE 420
CapH (A-503083) 312:HATSTHQVWVRIQDAEQTRDFRSKYLVDHGIRVNVQVDLPLGPPVLRLLGVNLTFLGGHE 371
LipK (A-90289) 318:GYTDTHQVWVVDGVAAYALSNRLEAGGIRVNVQVDLPLGPPVLRLLGSNEVTFEGAGP 377
.....*
Orf14 (A-500359) 421:AAVHALAEFFGNARAGVRRDGDGARRVCEQSGPPFYFAEFS----- 461
CapH (A-503083) 372:AAVHALAEFFSHARDGVRDGGGRVREQYGGPFFVFEFS----- 412
LipK (A-90289) 378:QAIEELAGALVTARERA-LGPRTVHEIRGRFGAPFYTFEKLKVEAGL 424
.....*

```

### Primer Sets

- A** is primer combination of DS-F1 and DS-R1,
- B** is of DS-F1 and DS-R2,
- C** is of DS-F2 and DS-R1,
- D** is of DS-F2 and DS-R2,
- E** is of DS-F3 and DS-R1,
- F** is of DS-F3 and DS-R2.

**Set B was used for 1<sup>st</sup> screening**  
**Set A and C were used for 2<sup>nd</sup> screening**



Specific for translocase I inhibitor producers

- Lane 1, *Streptomyces coelicolor* A3(2) ATCC BAA-471;
- Lane 2, *Streptomyces avermitilis* ATCC 31267;
- Lane 3, *Streptomyces gieseus* IFO 13350;
- Lane 4, *Saccharopolyspora erythroa* NRRL 3887;
- Lane 5, *Streptomyces lividans* TK21.
- Lane 6, *Streptomyces* sp. SANK 60405, A-90289 producer;
- Lane 7, *Streptomyces* sp. SANK 60704, A-97065 producer;
- Lane 8, *Streptomyces arenae* NRRL 2377, A-84830 producer.
- Lane 9, *Streptomyces griseus* SANK 60196, A-500359 producer;
- Lane 10, *Streptomyces* sp. SANK 62799, A-503083 producer;
- Lane 11, *Amycolatopsis* sp. SANK 60206, A-102395 producer.

Reaction Components	
0.5 M	betaine
X1	Gotaq
0.4 μM	forward primer
0.4 μM	reverse primer
15%	crude genome extracted by InstaGene D.W.

**Reaction Condition**  
 35 cycles of [94°C for 30 sec, 45°C for 30 sec and 72°C for 60 sec]

## III. PCR-based Screening

~2500 strains

1st ↓ rarely-explored actinomycetes isolated from soil

29

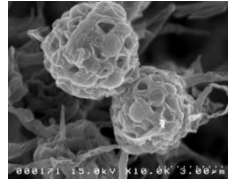
2nd ↓

6

Sequence Check

1

*Sphaerisporangium* sp. SANK 60911



```

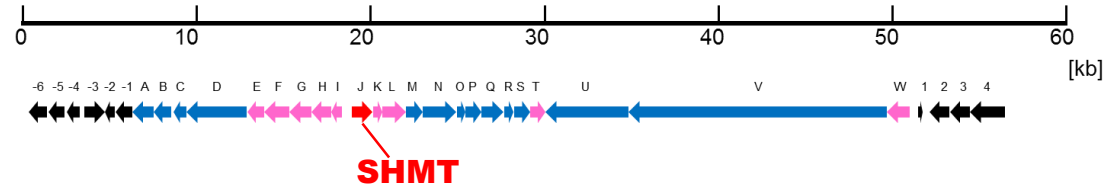
Orf14 (A-500359) 1  SFLA LPLS FNNRYFFNDLDPGFWFRGGCVKVTETLARGHLSLRRSPHVNE 60
CapH (A-503083) 1  KSPLA LPLS FNNRYFFNDLDPGFWFRGGCVKVTETLARGHLSLRRSPHVNE 60
LipK (A-90289) 1  KSPLA LPLS FNNRYFFNDLDPGFWFRGGCVKVTETLARGHLSLRRSPHVNE 59
HIT SHMT 1  SFLA LPLS FNNRYFFNDLDPGFWFRGGCVKVTETLARGHLSLRRSPHVNE 60

Orf14 (A-500359) 61  PISGLSANMVAAGLGGVCSFVWSIDANSGGHYAFAGMARLGGFEPVLPFPRVYDVE 120
CapH (A-503083) 61  PISGLSANMVAAGLGGVCSFVWSIDANSGGHYAFAGMARLGGFEPVLPFPRVYDVE 120
LipK (A-90289) 60  PISGSANMILTVAALDFVCSFVWSIDQNSGGHYAFALGLLGNRRLLNCKDEVDVE 118
HIT SHMT 61  PISGLSANMVAAGLGGVCSFVWSIDGHWGGHYAFALVPLGLRGNVLPFPRVYDVE 120

Orf14 (A-500359) 121  QRFQGLLRERTFQVLYLDLQNSRHEDESRVABLLKEYSFFSLHLVDSHTMELLGSAI 180
CapH (A-503083) 121  QRFQGLLRERTFQVLYLDLQNSRHEDESRVABLLKEYSFFSLHLVDSHTMELLGSAI 180
LipK (A-90289) 119  SFLRSLRGGVYDQVAVVVFDFRLEQVDEVSSEVSLRSHLLELLE 178
HIT SHMT 121  SFLRSLRGGVYDQVAVVVFDFRLEQVDEVSSEVSLRSHLLELLE 180

Orf14 (A-500359) 181  GNPLDAGAITGGSTHRKSPGPHKGVLFTRSESHQRLEKQOFTMLSS 228
CapH (A-503083) 181  SNPLDAGAITGGSTHRKSPGPHKGVLFTRSESHQRLEKQOFTMLSS 228
LipK (A-90289) 179  ANPLDAGAITGGSTHRKSPGPHKGVLFTRSESHQRLEKQOFTMLSS 226
HIT SHMT 181  FNPLDAGAITGGSTHRKSPGPHKGVLFTRSESHQRLEKQOFTMLSS 228
    
```

## IV. Sequence Analysis



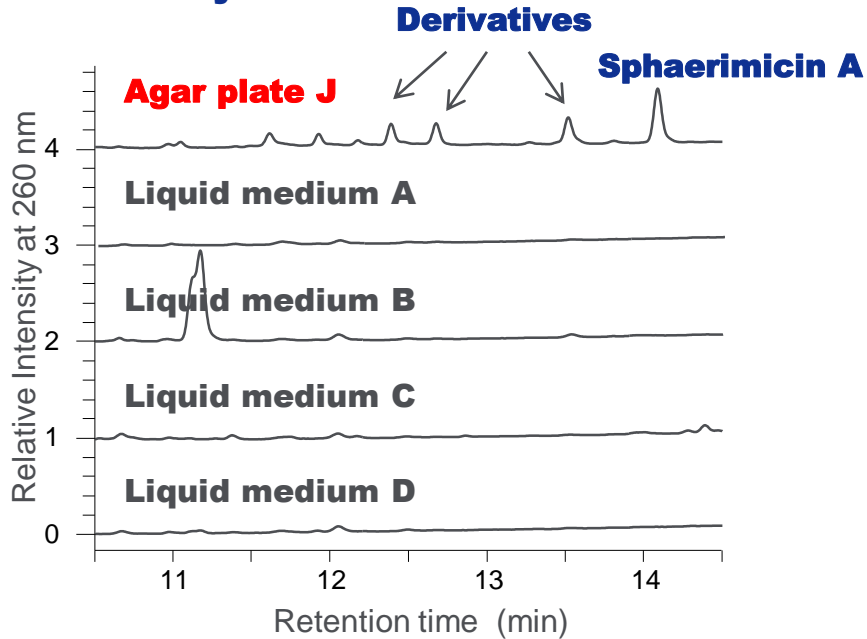
SphA	Glycosyl transferase group 1	SphM	Sucraseferredoxin family protein
SphB	Arylsulfatase	SphN	Transketolase
SphC	Phosphoesterase	SphO	MmgE/PrpD family protein
SphD	ABC transporter	SphP	Aldo/keto reductase
SphE	Dioxygenase	SphQ	Diaminopimelate decarboxylase
SphF	Pyrimidine-nucleoside phosphorylase	SphR	Hypothetical Protein
SphG	Aminotransferase	SphS	F420-dependent oxidoreductase
SphH	Glycosyltransferase	SphT	Nucleotidyltransferase
SphI	Nucleotidyltransferase	SphU	Type I polyketide synthase
SphJ	Serine hydroxymethyltransferase	SphV	Type I polyketide synthase
SphK	β-hydroxylase	SphW	Non-ribosomal peptide synthetase
SphL	Aminotransferase		

**There were several ORFs (pink) of which homologs are involved in other translocase I inhibitor biosynthesis**

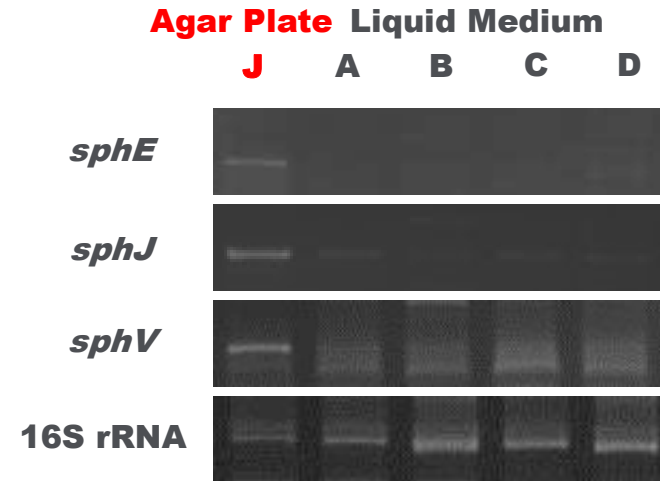
**Highly homologous to known SHMTs!**

## V. Cultivation and Activation

### HPLC Analysis

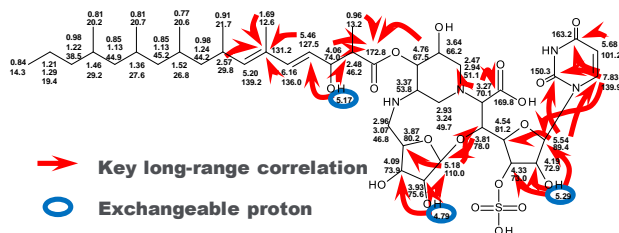


### RT-PCR Analysis

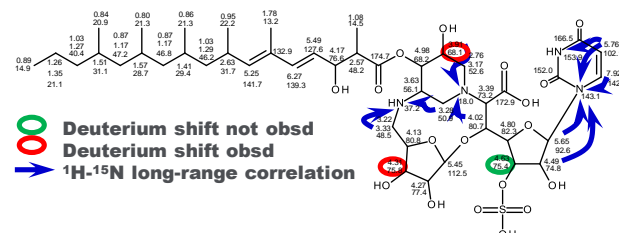


**Only when *Sphaerisporangium* sp. SANK 60911 was cultivated on agar plate, products with the characteristic absorption at 260 nm and the expression of some biosynthetic genes were detected**

## VI. Structural Elucidation



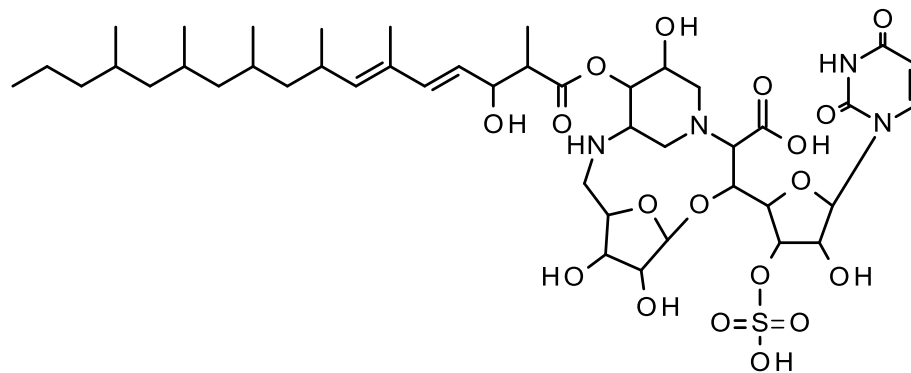
**Sphaerimicin A in DMSO- $d_6$**



**Sphaerimicin A in  $\text{CD}_3\text{OD}$**

***O*-sulfate group substituted at C-3'**

### Planar structure of sphaerimicin A



**Novel polyketide-nucleoside hybrid antibiotic with objective uridine moiety and unique piperidine ring system**

ご清聴有難うございました